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INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

<b>(51) International Patent Classification <sup>5</sup> :</b> <b>A61K 35/00, 35/23, 37/00</b>	<b>A1</b>	<b>(11) International Publication Number:</b> <b>WO 92/04033</b> <b>(43) International Publication Date:</b> 19 March 1992 (19.03.92)
<b>(21) International Application Number:</b> PCT/US91/06105 <b>(22) International Filing Date:</b> 27 August 1991 (27.08.91)  <b>(30) Priority data:</b> 575,150 30 August 1990 (30.08.90) US 671,517 19 March 1991 (19.03.91) US  <b>(71) Applicant:</b> THE GENERAL HOSPITAL CORPORATION [US/US]; Office of Technology Affairs, Thirteenth Street, Building 149, Suite 1101, Charlestown, MA 02129 (US).  <b>(72) Inventor:</b> FAUSTMAN, Denise ; 74 Pinecroft Road, Weston, MA 02193 (US).		<b>(74) Agent:</b> CLARK, Paul, T.; Fish & Richardson, 225 Franklin Street, Boston, MA 02110 (US).  <b>(81) Designated States:</b> AT (European patent), AU, BE (European patent), CA, CH (European patent), DE (European patent), DK, DK (European patent), ES (European patent), FI, FR (European patent), GB (European patent), GR (European patent), IT (European patent), JP, LU (European patent), NL (European patent), NO, SE (European patent).  <b>Published</b> <i>With international search report.</i>
<b>(54) Title:</b> METHODS FOR INHIBITING REJECTION OF TRANSPLANTED TISSUE  <b>(57) Abstract</b>  A method for inhibiting rejection by a recipient animal of a transplanted tissue, said method comprising modifying, eliminating, or masking an antigen which, when present on the surface of a cell of said tissue, is capable of causing a T-lymphocyte-mediated response in said animal, to inhibit antigen-mediated interaction between said cell and a T-lymphocyte of said animal without causing lysis of said cell.		

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METHODS FOR INHIBITING REJECTION OF TRANSPLANTED TISSUEBackground of the Invention

This invention relates to transplantation of tissues, e.g., islet cells, muscle cells, and whole  
5 organs, into hosts in need of such tissues, e.g., patients who have or are at risk of developing diabetes mellitus, have muscular dystrophy, or are in need of an organ transplant.

Diabetes mellitus is a prevalent degenerative  
10 disease, characterized by insulin deficiency, which prevents normal regulation of blood glucose levels, and which leads to hyperglycemia and ketoacidosis.

Insulin, a peptide hormone, promotes glucose utilization, protein synthesis, formation and storage of  
15 neutral lipids, and the growth of some cell types. Insulin is produced by the  $\beta$  cells within the islets of Langerhans of the pancreas. Early-onset diabetes (10-20% of cases) is caused by an auto-immune reaction that causes complete destruction of  $\beta$  cells. Adult-onset  
20 diabetes has a number of causes, but in most cases the  $\beta$  islet cells are defective in secretion of insulin.

Insulin injection therapy, usually with porcine or bovine insulin, prevents severe hyperglycemia and ketoacidosis, but fails to completely normalize blood  
25 glucose levels. While injection therapy has been quite successful, it fails to prevent the premature vascular deterioration that is now the leading cause of morbidity among diabetics. Diabetes-related vascular deterioration, which includes both microvascular  
30 degeneration and acceleration of atherosclerosis, can eventually cause renal failure, retinal deterioration, angina pectoris, myocardial infarction, peripheral neuropathy, and arteriosclerosis.

Recently, cloning of the human insulin-encoding  
35 gene has allowed large scale production of human insulin,

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which has begun to replace bovine insulin and porcine insulin as the treatment of choice. Use of human insulin has eliminated some of the problems associated with other forms of insulin, including antibody-mediated insulin resistance and allergic reactions resulting from the slightly different structures of non-human insulins. Despite these advantages, treatment with human insulin does not prevent vascular deterioration.

Insulin delivery pumps have been developed which administer varying doses of insulin based on activity, diet, time of day, and other pre-programmed factors. While such devices improve blood sugar control, they also do not prevent vascular deterioration.

Surgical transplantation of part or all of the pancreas is thought to be potentially the best treatment for diabetes. Successful transplantation is difficult, however, because the pancreas is a fragile and complicated organ, and it is impossible for a human donor to give only a portion of it; the only practicable source is a deceased donor. Further, only a small portion of the pancreas, the  $\beta$  cells of the islet of Langerhans, produce insulin; the remainder of the pancreas presents a potent target for transplant rejection. Transplantation of just the islets of Langerhans is a desirable goal, as they continue to secrete appropriate amounts of insulin in response to nutritional signals even when isolated from the rest of the pancreas.

A major problem associated with transplantation therapy as a treatment for diabetes is that current regimes require life-long administration of immunosuppressive drugs. These drugs can cause increased susceptibility to infection, renal failure, hypertension, and tumor growth.

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Despite these serious complications, islet transplantation has been successfully performed in experimental animals. Successful transplantation in rodents has been shown to restore normal blood glucose regulation and prevent further vascular deterioration. The broader application of allografts and xenografts (inter-species grafts) as a therapy for diabetes depends on preventing transplant rejection. It has long been known that culturing islets prior to transplantation decreases immunogenicity and increases transplant survival (Lacy et al (1979) Science 204\_312; Lafferty et al. (1975) Science 188:259). It is thought that long term culturing removes the Ia-bearing passenger lymphoid cells, which are a primary stimulus for cell-mediated immunity and graft rejection. Faustman et al. (J. Exp. Med. 151:1673, 1980) found that islet cells lack Ia antigenic determinants and express class I antigen on their surfaces. This allowed Faustman et al. (Proc. Natl. Acad. of Sci. USA 78:5156, 1981) to develop a regime that used donor-specific anti-Ia serum and complement to destroy Ia bearing lymphoid cells in islets, and allowed transplantation across a major histocompatibility barrier into non-immunosuppressed diabetic mice.

25                   Summary of the Invention

The invention features a method for inhibiting rejection by a recipient animal of a transplanted tissue. The method involves modifying, eliminating, or masking an antigen which, when present on the surface of a cell of the tissue, is capable of causing a T-lymphocyte-mediated response in the animal; modification, elimination, or masking of the antigen inhibits antigen-mediated interaction between the cell and a T-lymphocyte of the animal, without causing lysis of the cell.

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Where cells of the tissue for transplantation (the "donor" tissue) bear on their surfaces HLA class I antigens (members of one of the classes of major histocompatibility complex antigens), these antigens cause cytotoxic T-cell activation in recipients, terminating in donor cell lysis after several sequential activation steps. The cascade is initiated by non-specific conjugate formation between the CD8 receptor on host cytotoxic T-cells and the HLA class I antigens on the donor cell. Conjugate formation is followed by T-cell-mediated lysis, resulting in donor cell death. This lytic process can result in rejection even in intra-species transplantation. According to the invention, this problem is addressed by masking, modifying, or eliminating of the HLA class I antigens on the donor cells, so that the CD8-HLA class I antigen interaction which initiates the lytic cascade cannot occur.

As will be explained in more detail below, any T-cell receptor-interactive antigens on the surfaces of donor cells can advantageously be modified, eliminated, or masked according to the invention. The invention thus permits not just intra-species transplantation of tissues and organs, but xenografts as well, opening up the possibility of "farming" of donor organs and tissues in non-human animals for transplantation into human patients.

Preferred masking agents are  $F(ab')_2$  fragments of antibodies to donor cell antigens; these fragments, while being capable of forming immune complexes with the antigen and thus preventing antigen-T-cell interaction, do not, because they have had the Fc portion of the antibody removed, fix complement and bring about cell lysis. It has been found that, even though one might not expect such  $F(ab')_2$  fragments to bind tightly enough to

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permanently mask antigenic sites on donor cells, long-term host acceptance of such treated tissues can be achieved.

As will be explained in greater detail below, rejection-inducing surface antigens on cells of donor tissues can, in addition to being masked, be modified, e.g., by "capping", or wholly or partially eliminated by genetic manipulation, either in transgenic animals used as a source of donor tissue, or in culture.

Other features and advantages of the invention will be apparent from the following description of the preferred embodiments thereof, and from the claims.

#### Description of the Preferred Embodiments

The drawings are first briefly described.

#### Drawings

Fig. 1 is a set of graphs illustrating the expression of HLA-class I (W6/32), CD29 (4B4), CD54 (ICAM-1) and CD58 (LFA-3) on freshly isolated 99-97% pure whole human islets of Langerhans by indirect immunofluorescence and flow cytometry. A. Human islets were positive at 36% with W6/32 antibody (\_\_\_\_). B. Human islets were negative for CD29 with 9% expression (\_\_\_\_). C. Human islets in this clean islet preparation were virtually negative for ICAM-1 with 14% expression (\_\_\_\_). D. Human islets were negative for LFA-3 expression with 10.2% of the cells positive. Background goat anti-mouse FITC expression was 9% (----) for this experiment. An open gate with exclusion of dead cells and debris was used for flow cytometry. As expected, islet preparations contaminated with large amounts of fibroblast overgrowth or endothelial cells (purity 60-75%) were positive for low levels of LFA-3 and ICAM.

Figure 2 is a series of photographs showing histologic analysis of human islets transplanted under

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the kidney capsule of Balb/c recipients A. Photomicrograph of human islet xenograft 30 days after transplant with pre-transplant treatment with HLA class I F(ab')<sub>2</sub> fragments (W6/32). This aldehyde fuscine stain (X100) shows well-granulated islets under the kidney capsule. B. Photomicrograph of human islet xenograft 200 days after transplant with pretransplant treatment with HLA class I F(ab')<sub>2</sub> fragments (W6/32). This aldehyde fuscine stain (X100) shows well-granulated islets under the kidney capsule. C. A control Balb/c mouse was transplanted with untreated fresh human islets and then killed at day 30. This characteristic photomicrograph shows the absence of donor islets and the presence of subcapsular fibrosis demonstrating the former area where the islets were transplanted. D. Aldehyde fuscine stain of a mouse islet in the mouse pancreas demonstrating the characteristic purple granulation of healthy beta cells.

#### Donor Tissue Preparation

Before describing in detail specific examples of the invention, there is a brief discussion of some parameters of the invention.

#### Donor Tissue

In addition to permitting transplantation of islet cells, the invention can facilitate transplantation of any other tissue or organ, e.g., kidney, heart, liver, lung, brain, and muscle tissue.

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Antigens to be Masked, Modified, or Eliminated

The invention can be used to mask, modify, or eliminate any host T-cell-interactive antigen on any of the cells of the donor tissue. In addition to HLA class I antigens, which are found on all parenchymal cells, including islet cells, other important donor cell antigens known to interact with host T-cells to bring about rejection are LFA-3 and ICAM-1; these react, respectively, with the host T-cell receptors CD2 and LFA-1. Both LFA-3 and ICAM-1 are found on endothelial cells which make up blood vessels in transplanted organs such as kidney and heart. Masking, altering, or eliminating these antigens will facilitate transplantation of any vascularized implant, by preventing recognition of those antigens by CD2+ and LFA-1+ host T-lymphocytes. Further, masking, altering, or eliminating a particular donor cell antigen may render more than one donor cell-type less susceptible to rejection. For example, not only do parenchymal cells such as islet cells bear HLA class I antigens, but passenger lymphocytes bear such antigens as well, and if such lymphocytes are present in a donor tissue preparation, removal of an HLA class I antigen or treatment of the tissue preparation with an HLA class I antigen masking agent will render those lymphocytes less antigenic.

The antigens HLA class I, LFA-3, and ICAM-1 are well-characterized, and antibodies to these antigens are publicly available, and can be made by standard technique. For example, anti-ICAM-1 can be obtained from AMAC, Inc., Maine; hybridoma cells producing anti-LFA-3 can be obtained from the American Type Culture Collection, Rockville, Maryland.

Where the donor tissue to be transplanted bears more than one T-cell-interactive antigen, two or more

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treatments, e.g., two or more masking agents, may be used together. Alternatively, a polyclonal antisera generated against the donor tissue may be used to mask multiple cell surface antigens of the donor tissue.

5        Non-Lytic Masking Agents

Generally, the invention can employ three categories of masking agents: (1) antibodies or fragments or derivatives thereof; (2) soluble fragments or analogs of antigen-specific host T-cell receptors; and  
10        (3) synthetic organic molecules which mimic the antigen binding properties of T-cell receptors.

Antibodies, the currently most preferred masking agents, can be used either as one or more antigen-specific preparations, or as whole donor organ or tissue  
15        antisera preparations. In either case, it is necessary that the preparations be unable to fix complement and bring about donor cell lysis. Complement fixation can be prevented by deletion of the Fc portion of the antibody, by using an antibody isotype which is not capable of  
20        fixing complement, or, less preferably, by using a complement fixing antibody in conjunction with a drug which inhibits complement fixation.

Individual antigen-specific antibodies can be made by standard procedures, including immunization of an  
25        animal, e.g., a mouse, with the antigen to be masked, followed by hybridoma preparation and antibody screening according to standard methods. Alternatively, whole donor antisera can also be used. For example, where the donor tissue is derived from a pig, whole pig antisera  
30        are produced by immunization of a mouse with pig donor tissue or pig lymphocytes, followed by screening for antibodies which block human T-lymphocyte adhesion to pig donor cells.

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an alternative to antibodies or antibody fragments, masking can be effected by use of soluble host T-cell receptor which competitively inhibits binding of those T-cells to donor tissue cell antigens, by occupying the antigenic site on the tissue which would otherwise interact with the host T-cells. T-cell molecules or proteins, e.g., CD8, CD2, and LFA-1, are well characterized proteins generally having an extracellular domain, a transmembrane region, and a cytoplasmic domain which bend to target cell ligands. Soluble T-cell receptor protein fragments can be made by standard recombinant DNA procedures, in which the DNA encoding the transmembrane and cytoplasmic domains is deleted, and the extracellular domain DNA is expressed in recombinant cells to produce soluble recombinant protein.

#### Capping

Capping is a term referring to the use of antibodies to cause aggregation and inactivation of surface antigens. First, the tissue is contacted with an antibody specific for the antigen, so that antigen-antibody immune complexes are formed. The next step is contacting the tissue with a second antibody capable of forming immune complexes with the first antibody, so that the first antibody is aggregated to form a cap at a single location on the cell surface. The technique is well known, and has been described, e.g., in Taylor et al. (1971), Nat. New Biol. 233:225-227; and Santiso et al. (1986), Blood, 67:343-349. In the case of cells, e.g., islet cells, bearing HLA class I antigens, the first step is to incubate the cells with antibody (e.g., W6/32 antibody, described below) to HLA class I, and then to incubate with antibody to the donor species, e.g., goat anti-mouse antibody, to bring about aggregation.

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Transgenic Animals with Decreased  
HLA Class I Expression

As an alternative or an adjunct to masking surface antigens on cells of donor tissues prior to

5   transplantation, such tissues can be grown in transgenic animals which have been genetically altered so that surface antigen expression is diminished. Such transgenic animals can be made by standard transgenic techniques, employing genes which delete or inactivate

10   the gene encoding the target antigen, or delete or inactivate a gene necessary for its expression on the cell surface, by homologous recombination.

For example, in the case of HLA class I expression, homologous recombination can be used either

15   to delete or inactivate the HLA class I molecule itself, or to inactivate or delete a companion molecule necessary for its surface expression. The HLA class I molecule is a protein composed of a 32Kd and a 45Kd chain, associated with another protein,  $\beta$ -2 microglobulin. The highly

20   conserved  $\beta$ -2 microglobulin protein is believed to function as a carrier molecule which facilitates class I assembly in plasma membranes.

Inhibition of class I expression on the surfaces of cells, e.g., islet cells, can thus be achieved either

25   by deletion or inactivation of one of the HLA class I chains, or by deletion or inactivation of the carrier  $\beta$ -2 microglobulin molecule. Disruption of  $\beta$ -2 microglobulin expression in transgenic animals resulting in decreased HLA class I expression has been carried out

30   by several groups (Koller and Smithies (1989), PNAS USA 86:8932-8935; Zijlstra et al. (1990) Nature, 344:742-746; Doetschman et al. (1987) Nature, 51:503-512).

In Vitro Methods to Decrease HLA Class I  
Expression

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A number of oncogenic viruses have been demonstrated to decrease HLA class I expression in infected cells; Travers et. (1980) Int'l. Symp. on Aging in Cancer, 175-180; Rees et al. (1988) Br. J. Cancer, 57:374-377. In addition, it has been demonstrated that this effect on HLA class I expression can be achieved using fragments of viral genomes, in addition to intact virus. Transfection of cultured kidney cells with fragments of adenovirus causes elimination of surface HLA class I antigenic expression; Whoshi et al. (1988) J. Exp. Med. 168:2153-2164. For purposes of decreasing HLA class I expression on the surfaces of donor cells, e.g., islet cells, viral fragments, which are non-infectious, are preferable to whole viruses, which could cause complications. Other viruses and viral fragments could be used to decrease expression of other surface antigens on other types of donor cells, as well as decreasing expression of HLA class I expression on parenchymal cells such as islet cells.

20 Local Blockage of Recipient T-Cell Receptors  
With Secreted Donor Antigens

The transplantation inhibition strategies discussed above all involve altering the donor tissue such that surface antigens on donor tissue cells which are recognized as foreign by receptors on recipient T-cells are eliminated, modified, or masked. An alternative strategy is to modify the donor tissue in a different way, which brings about blockage of the host T-cell receptors by antigen secreted by donor cells. For example, in the case of donor tissue containing parenchymal cells bearing surface HLA class I antigen, rather than masking the antigen, those cells can be transfected with DNA encoding soluble antigen, which is secreted and which competitively binds to the CD8

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receptor on the T-lymphocytes of the recipient which would otherwise bind to membrane-bound HLA class I antigen on the donor tissue cells. The techniques for carrying out this procedure will be analogous to methods used by other workers to bring about secretion of a recombinant protein in concert with insulin secretion; Lo et al. (1988) Cell, 53:159-168; Adams et al. (1987) Nature, 325:223-228. Adams et al. achieved SV40 T antigen synthesis in islet cells in concert with insulin production. HLA class I antigen expression and secretion could be coupled to insulin production and secretion by placing the gene for one or both subunits of HLA class I antigen under the control of insulin gene regulatory sequences. Insulin secretion thus will result in simultaneous expression and secretion of HLA class I antigen. This strategy has the advantage of causing secretion of HLA class I antigen only from islet cells, in tissue which may contain other cell types as well; none of the other cells present produce and secrete insulin. In addition, this approach confines the soluble HLA class I antigen to the localized region where it is needed, i.e., in the area immediately surrounding the transplanted islet cells.

The following specific examples are for illustration purposes only, and are not intended to limit the scope of the invention.

#### Example 1

This example involves xenogeneic transplantation of HLA class I positive human islet cells into nonimmunosuppressed Balb/c mouse recipients. Freshly isolated human islets were pretreated prior to transplantation with whole monoclonal antibody or F(ab')<sub>2</sub> monoclonal antibody fragments to conceal ("mask") donor antigens. F(ab')<sub>2</sub> fragments lack the Fc antibody region,

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thus circumventing complement-mediated killing after antigen binding. Intact immunoglobulin was used as a control. Human islets were treated with relevant HLA class I monoclonal antibody (W6/32) (American Tissue Culture Society) (Barnstable et al. (1979) Cell, 14:9-20) or irrelevant CD29 monoclonal antibody (Coulter Corporation, Hialeah, FL). Clean human islet preparations, free of contaminating endothelial and fibroblast overgrowth, are negative for ICAM-1 expression, negative for CD29 expression, have low LFA-3 expression, and are positive for HLA class I antigens (Figure 1). Therefore, islets, unlike other cytotoxic T-lymphocyte targets, lack the prominent expression of the two important adhesion epitopes LFA-3 and ICAM-1, and there is little need to protect these adhesion epitopes from T-cell binding.

F(ab')<sub>2</sub> fragments were generated using an immobilized pepsin. Purified antibody added at 20 mg/ml in pH 4.7 digestion buffer was digested for 4.5 hours for CD29 antibody and 4.0 hours for W6/32 antibody (HLA class I) at 37°C in a shaker water bath (Pierce Chemical, Rockford, IL). The crude digest was removed from the pepsin and immediately neutralized with pH 7.0 binding buffer. This antibody mixture was applied to an immobilized Protein A column and the eluate collected for the F(ab')<sub>2</sub> fragments. Dialysis against PBS for 24 hours using 50,000 M.W. cutoff tubing was then performed to rid the digest of contaminating Fc fragments. CHAPPS was added to the dialysis bag at a concentration of 10 mM. The completeness of the digest and purification of the F(ab')<sub>2</sub> digests were monitored by silver staining of 15% SDS gels. Final purification of the fragments was achieved by HPLC using a Superose 12 column (Pharmacia, Upsala, Sweden).

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F(ab')<sub>2</sub> fragments or whole antibodies were incubated with human islets at a concentration of 1 µg of antibody for approximately 1 x 10<sup>6</sup> islet cells for 30 minutes at room temperature. After incubation, the treated or untreated islets were washed once in Hanks buffer containing 2% FCS and then immediately transplanted under the kidney capsule by syringe injection. The human islets used were transplanted within 4 days after isolation. Ten to twelve week old Balb/c female mice (The Jackson Laboratories, Bar Harbor, Maine) were transplanted with 2200-4500 human islets. At 30 or 200 days post transplantation the mice were sacrificed by cervical dislocation and the kidney containing the transplanted tissue was surgically removed and immediately fixed in Bouin's solution.

The results of the transplantation studies are summarized in Table I. W6/32 F(ab')<sub>2</sub> pretreatment of donor xenogeneic islets (HLA class I) resulted in complete islet xenograft survival in all 5 recipients evaluated at 30 days after transplantation (Group 1) as well as all 5 recipients evaluated at 200 days after transplantation (Group 2). The histology in all 10 mice revealed well-granulated islets under the kidney capsules (Figure 1A, 1B). Untreated human islets were promptly rejected by 7 days in this mouse model; histology in these mice showed massive lymphocytic infiltrates under the kidney capsules and no granulated islet cells. The HLA class I F(ab')<sub>2</sub> treated islet grafts (W6/32) were virtually free of adjacent lymphocyte deposits even at 200 days following transplantation (Figure 1B). Lymphocytic accumulations are a known characteristic of xenograft transplants prolonged with culture.

Balb/c recipients receiving islet grafts pretreated with whole HLA class I W6/32 antibody

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demonstrated no surviving islet tissue at 30 or 200 days after transplantation (Group 3, 4) (Table 1), indicating probable complement fixation and lysis by the whole, uncut antibody. Histology performed on these transplants revealed subcapsular kidney fibrosis at the transplantation site (Figure 1C). The coating of donor islets with irrelevant F(ab')<sub>2</sub> fragments directed at CD29 resulted in islet graft rejection by day 30 as well as day 200 (Group 5, 6). Intact, CD29 antibody also failed to prolong islet xenograft survival (Group 7, 8). The pretreatment of donor human islets with specific HLA class I F(ab')<sub>2</sub> antibody fragments (W6/32) and with irrelevant CD29 F(ab')<sub>2</sub> antibody fragments (CD29) resulted in graft survival in all five recipients at day 30 (Group 9) and all five recipients at day 200 (Group 10) as observed for HLA class I F(ab')<sub>2</sub> fragments alone. As expected, untreated human islets were absent at both the 30 day and 200 day evaluation time points (Group 11, 12). Only subcapsular fibrosis was present under the kidney capsule at day 30 (Figure 1C) and day 200 in these recipients.

The function of transplanted human islets was monitored by evaluating human insulin C' peptide levels at 30 and 200 days post transplantation (Table 2). All 20 recipients receiving W6/32 F(ab')<sub>2</sub> coated human islets or W6/32 F(ab')<sub>2</sub> and CD29 F(ab')<sub>2</sub> coated islets at day 30 had detectable human C' peptide levels markedly above background levels (Groups 1, 2, 9, 10) (p=.002). Human C' peptide levels were similarly detected at 200 days in all ten recipients receiving W6/32 F(ab')<sub>2</sub> antibody coated islets (Groups 2, 10) (p=.003). In contrast, all individuals in the control transplant groups had human C'

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peptide levels similar to background levels (Group 3, 4, 5, 6, 7, 8, 11, 12) ( $p=.98$ ).

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Example 2

This example involves the xenogeneic transplantation of rat insulinoma tumor cells (RIN) into nonimmunosuppressed Balb/c mouse recipients to investigate the possibility of graft specific tolerance with growth of a transplanted tissue pre-treated with polyclonal F(ab')<sub>2</sub>.

RIN tumor cells are an established rat insuloma tumor cell line (Meflasson et al., 1983 J. Biol. Chem. 258:2094-2097). Polyclonal mouse anti-RIN serum was produced and F(ab')<sub>2</sub> antibody fragments were generated as described above in Example 1. As expected, xenogeneic RIN cells (approx. 5,000 cells per recipient) transplanted under the kidney capsule of nonimmunosuppressed BALB/c mice were uniformly rejected when evaluated by histology with aldehyde fuchsin staining (n=4) (Table 3). In addition, pretreatment of RIN cells with intact polyclonal mouse anti-RIN antibody, without removal of the complement-fixing Fc region, prior to transplantation also failed to protect grafts from recipient mediated rejection (n=4). In contrast, pretreatment of RIN cells with F(ab')<sub>2</sub> fragments of mouse anti-RIN polyclonal antibodies allowed RIN cell survival at one, two, three, and four months after transplantation. Even though each BALB/c recipient received an equal number of cells at the time of transplantation, serial sections through the transplant site under the kidney capsules at different monthly intervals after transplantation revealed a noticeable increase in the mass of tumor tissue, suggesting tumor growth. In addition, the successfully transplanted RIN cells demonstrated mitosis on hematoxylin and eosin staining, confirming cell division and presumably the new expression of unmasked foreign antigens. The continued

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survival and expansion of a xenogeneic tumor cell line suggests the possible presence of graft induced tolerance in the recipients. Further proof of a state of graft tolerance was obtained by transplanting  $F(ab')_2$  coated RIN cells unilaterally into the right kidney of nonimmunosuppressed mice for 30 days prior to a secondary transplant of untreated RIN cells into the left kidney. At day 60 the four mice transplanted in this manner were sacrificed. The four untreated secondary transplants of insulinoma cells also demonstrated survival, confirming the suspected development of a systemic tolerant state sufficient for fresh tumor cell survival.

### Example 3

The effectiveness of  $F(ab')_2$  HLA class I antibody coating in preventing rejection of non-tumorigenic human liver cells in xenogeneic transplants was also investigated. Approximately 5,000 fresh human liver cells from the parenchymal tissue of the liver were injected into the subscapular space of the kidney capsules of nonimmunosuppressed mouse recipients. Histological examination using PAS staining of the subscapular sites indicated that all 5 transplant recipients of  $F(ab')_2$ - treated liver cells demonstrated easily located viable liver cells at the subscapular renal site 30 days after transplantation. As expected, untreated human liver cells were uniformly rejected in all five mice by day 30 after transplantation.

It is clear from the results of Examples 1 and 3 that the simple interruption of recipient T cell recognition by masking of foreign HLA class I determinants allows prolonged xenograft survival up to 200 days. This new strategy eliminates recipient treatment, thus preserving the immune response of the

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host so that it remains available for recognition of relevant pathogens.

The prolonged duration of recipient unresponsiveness to a viable tissue which eventually might lose the masking antibody or exhibit the ability to resynthesize new uncoated HLA class I determinants suggests that graft specific tolerance may stabilize these transplants. This is substantiated by the lack of large foci of lymphocyte infiltrates in my successful xenografts. This is consistent with my assumption that donor pretreatment of the graft with HLA class I antibody fragments coats class I antigens on transient donor dendritic cells as well as class I antigen on the parenchymal islet cells. With the passage of time post-transplantation, these antigen presenting cells which are potent graft rejection initiators may die off, as occurs with extended culture, thus gradually exposing the recipient to low levels of HLA class I antigens on non-antigen presenting cells.

20                   Other Embodiments

Other embodiments are within the following claims. For example, the procedures described above for treatment of islet cells and liver cells can be used to treat muscle cells for transplantation into patients with muscular dystrophy, as follows; muscle cells, like islet cells, bear rejection-stimulating HLA class I antigens, and also express class II antigens. Human donor muscle cells will be obtained by biopsy of living related donors or brain dead donors using a 14-16 gauge cutting trochar into a 1-2 inch skin incision. The fresh muscle plug will then be lightly digested into a single cell suspension using collagenase, trypsin and dispase at 37°C. Floating debris will be removed with a pipet and media washes and the viable cell pellet counted after

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centrifugation at 1000 rpm X 10 minutes. This cell count will then be used to calculate the amount of HLA class I and class II antibody fragments to add; treatment will be as described above for islet cells. Similarly, the invention will permit transplantation of cells, from a healthy individual or which have been genetically engineered, into recipients who have a deficiency for a particular cellular component. For example, individuals with hemophilia might be recipients of Factor VIII-producing liver cells from normal donors, or of cells which have been genetically engineered to secrete Factor VIII.

Another embodiment of the invention would be the transplantation into patients of whole organs (e.g. heart, lung, liver, kidney). A preferred organ masking pre-treatment procedure would involve perfusion of the donor organ with F(ab')<sub>2</sub> fragments of monoclonal antigen-specific antibodies or with polyclonal antisera generated against the organ tissue; perfusion is carried out using conventional techniques for perfusing donor organs with other fluids.

What is claimed is:

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Table 1: Transplantation outcome of masking of donor human xenografts with HLA class I (W6/32) or CD29 antibodies or F(ab')<sub>2</sub> fragments prior to grafting

Groups	Islet Tissue Treatment <sup>②</sup>	Days S/P Transplantation <sup>③</sup>	ACCEPTED/TOTAL <sup>④</sup>
1	W6/32 F(ab') <sub>2</sub>	30 days	8/8
2	W6/32 F(ab') <sub>2</sub>	200 days	5/5
3	W6/32 Antibody	30 days	0/5
4	W6/32 Antibody	200 days	0/5
5	CD29 F(ab') <sub>2</sub>	30 days	0/5
6	CD29 F(ab') <sub>2</sub>	200 days	0/5
7	CD29 Antibody	30 days	0/5
8	CD29 Antibody	200 days	0/5
9	W6/32 F(ab') <sub>2</sub> +CD29 F(ab') <sub>2</sub>	30 days	5/5
10	W6/32 F(ab') <sub>2</sub> +CD29 F(ab') <sub>2</sub>	200 days	5/5
11	None	30 days	0/5
12	None	200 days	0/5

<sup>①</sup> Days S/P transplantation represents the number of days after transplantation when BALB/c recipients of donor human islets were sacrificed.

<sup>②</sup> Clean human islet preparations express class I antigens but lack CD29 determinants.

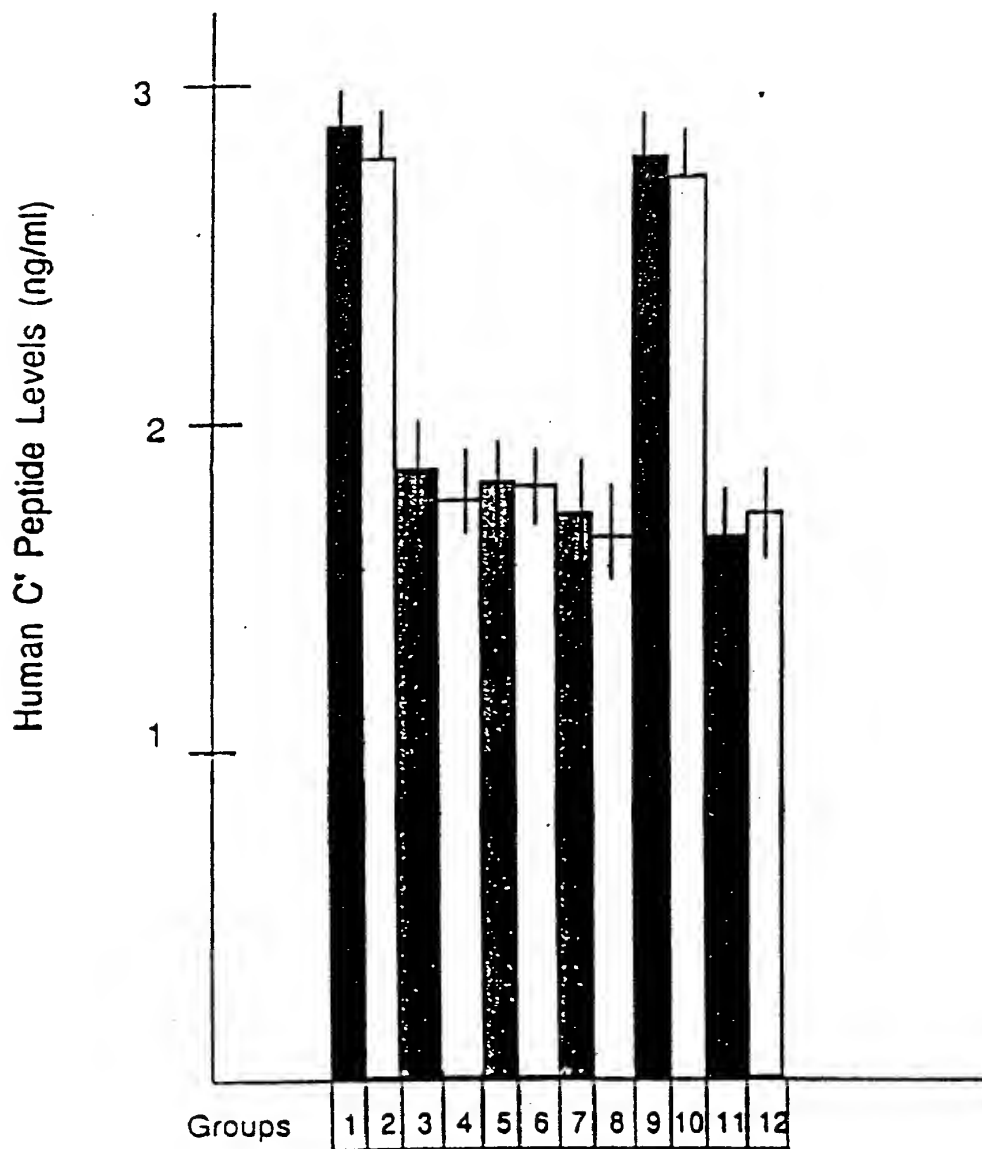
<sup>③</sup> The ratio represents the number of successful transplants to the number of transplants performed. Islet xenograft survival was evaluated with hematoxylin and eosin staining for the evaluation of lymphocyte infiltration and aldehyde-fuchsin staining for the detection of beta cells.

<sup>④</sup> Aldehyde-fuchsin stains well granulated beta cells purple hematoxylin and eosin stains lymphocytes black. Accepted in this manuscript represents easily located well granulated islets under the kidney capsule lacking lymphocyte foci. Rejection in this manuscript represents a transplant site with large lymphocyte accumulations and/or subcapsular fibrosis without islets.

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TABLE 2: Function of Human Islet Xenografts Evaluated by Human C' Peptide Levels



Group 1 W6/32 F(ab')<sub>2</sub>, d30  
 Group 2 W6/32 F(ab')<sub>2</sub>, d200  
 Group 3 W6/32 Antibody, d30  
 Group 4 W6/32 Antibody, d200  
 Group 5 CD29 F(ab')<sub>2</sub>, d30  
 Group 6 CD29 F(ab')<sub>2</sub>, d 200

Group 7 CD29 Antibody, d30  
 Group 8 CD29 Antibody, d200  
 Group 9 W6/32+CD29 F(ab')<sub>2</sub>, d30  
 Group 10 W6/32 + CD29 F(ab')<sub>2</sub>, d200  
 Group 11 No treatment, d30  
 Group 12 No treatment, d200

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TABLE 3 Transplantation of RIN Insulinoma cells into nonimmunosuppressed BABL/c recipients.

<u>DONOR RIN TREATMENT</u>	<u>HISTOLOGIC RESULTS AT AUTOPSY</u>
None	All 4 mice lack RIN cells at 1, 2, 3, and 4 months with the transplant site demonstrating subcapsular fibrosis.
Anti-RIN Antibody	All 4 mice lack RIN cells at 1, 2, 3, and 4 months with the transplant site demonstrating subcapsular fibrosis.
Anti-RIN F(ab') <sub>2</sub> fragments	All 4 mice demonstrate RIN cell survival at serial autopsy times of 1, 2, 3, and 4 months after transplantation. Slight but consistent lymphocytic infiltrates are visible at the transplant site

CLAIMS:

1           1. A method for inhibiting rejection by a  
2 recipient animal of a transplanted tissue, said method  
3 comprising modifying, eliminating, or masking an antigen  
4 which, when present on the surface of a cell of said  
5 tissue, is capable of causing a T-lymphocyte-mediated  
6 response in said animal, to inhibit antigen-mediated  
7 interaction between said cell and a T-lymphocyte of said  
8 animal without causing lysis of said cell.

1           2. The method of claim 1 wherein said inhibiting  
2 comprises masking said antigen by treating said tissue  
3 with a non-lytic masking agent which is capable of  
4 forming a complex with said antigen on said cell.

1           3. The method of claim 1 wherein said inhibiting  
2 comprises modifying said antigen by capping.

1           4. The method of claim 1 wherein said inhibiting  
2 comprises eliminating said antigen by inhibiting  
3 expression of said antigen on said cell.

1           5. The method of claim 4 wherein said antigen is  
2 an HLA class I antigen and said expression inhibiting  
3 comprises transfection of said cell with a fragment of a  
4 viral genome which decreases HLA class I expression.

1           6. The method of claim 1 wherein said inhibiting  
2 comprises eliminating said antigen by harvesting said  
3 tissue from a transgenic animal which has diminished  
4 capacity to express said antigen on the surface of said  
5 cell.

1           7. The method of claim 6 wherein said antigen is  
2 an HLA class I antigen.

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1           8. The method of claim 7 wherein said transgenic  
2 animal exhibits decreased beta-2 microglobulin  
3 expression.

1           9. The method of claim 7 wherein said transgenic  
2 animal exhibits decreased HLA class I antigen expression.

1           10. The method of claim 1 wherein said cell is an  
2 islet cell.

1           11. The method of claim 1 wherein said tissue and  
2 said animal are of different species.

1           12. The method of claim 1 wherein said tissue and  
2 said animal are of the same species.

1           13. The method of claim 1 wherein said antigen is  
2 an HLA class I antigen.

1           14. The method of claim 2 wherein said masking  
2 agent comprises an antibody or fragment thereof.

1           15 The method of claim 14 wherein said antibody  
2 is monoclonal.

1           16. The method of claim 14 wherein said antibody  
2 comprises a polyclonal antisera against said tissue.

1           17. The method of claim 14 wherein said antibody  
2 is an F(ab')<sub>2</sub> fragment.

1           18. The method of claim 2 wherein said antigen is  
2 an HLA class I antigen and a cytotoxic CD8+ lymphocyte of

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3 said animal is inhibited, by said masking agent, from  
4 interacting with said HLA class I antigen on said cell.

1 19. The method of claim 2 wherein said antigen is  
2 an LFA-3 molecule and a cytotoxic CD2+ lymphocyte of said  
3 animal is inhibited, by said masking agent, from  
4 interacting with said LFA-3 molecule on said cell.

1 20. The method of claim 2 wherein said antigen is  
2 an ICAM-1 molecule and a cytotoxic LFA-1+ lymphocyte of  
3 said animal is inhibited, by said masking agent, from  
4 interacting with said ICAM-1 molecule on said cell.

1 21. A tissue sample for transplantation into an  
2 animal, said tissue containing cells of a type normally  
3 bearing a surface antigen capable of causing a T-  
4 lymphocyte-mediated response in said animal, wherein said  
5 antigen on cells of said tissue sample is modified,  
6 masked, or has been eliminated to decrease said T-  
7 lymphocyte-mediated response.

1 22. The tissue sample of claim 21 wherein said  
2 cells comprise genetically engineered cells with  
3 increased capacity to express a cellular component.

1 23. The tissue of claim 21 wherein said antigen  
2 is an HLA class I antigen.

1 24. The tissue of claim 21 wherein said antigen  
2 is an HLA class II antigen.

1 25. The tissue of claim 21 wherein said antigen  
2 is masked with an F(ab')<sub>2</sub> fragment of an antibody.

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1           26. The tissue of claim 26 wherein said  $F(ab')_2$   
2 fragment comprises a polyclonal antisera generated  
3 against said tissue.

1           27. The tissue of claim 21 wherein said cells are  
2 islet cells.

1           28. The tissue of claim 21 wherein said cells are  
2 muscle cells.

1           29. The tissue of claim 21 wherein said cells are  
2 liver cells.

3           30. The tissue of claim 21 wherein said cells are  
4 neuronal cells.

1           31. The tissue of claim 21 wherein said tissue  
2 comprises heart tissue.

1           32. The tissue of claim 21 wherein said tissue  
2 comprises lung tissue.

1           33. The tissue of claim 21 wherein said tissue  
2 comprises liver tissue.

1           34. The tissue of claim 21 wherein said tissue  
2 comprises kidney tissue.

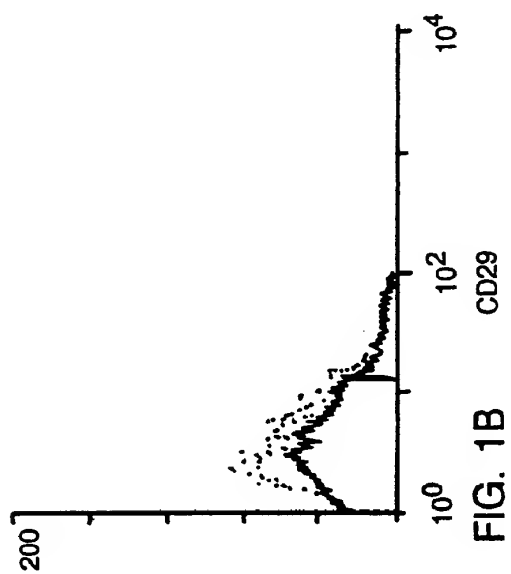
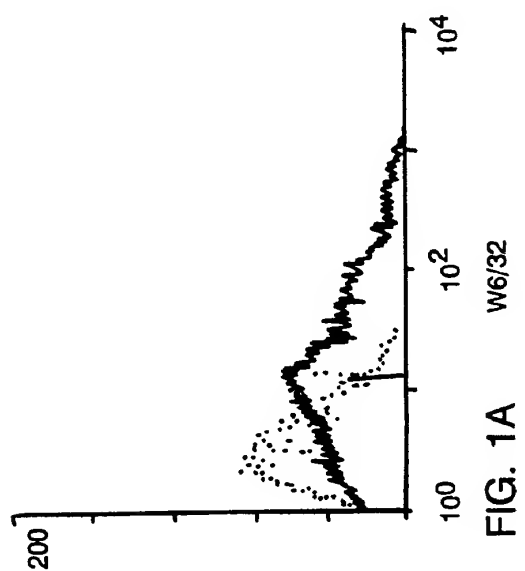
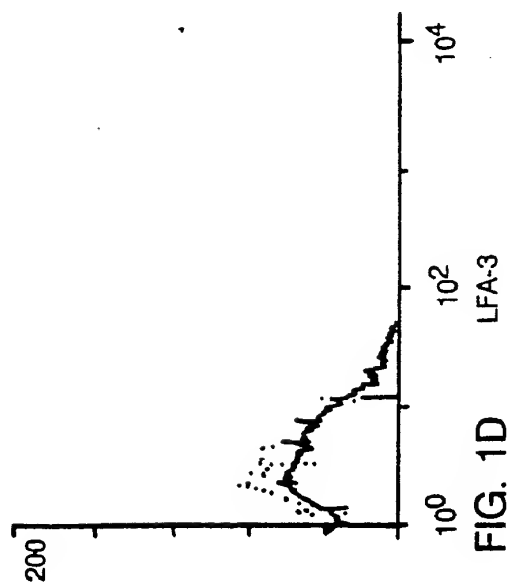
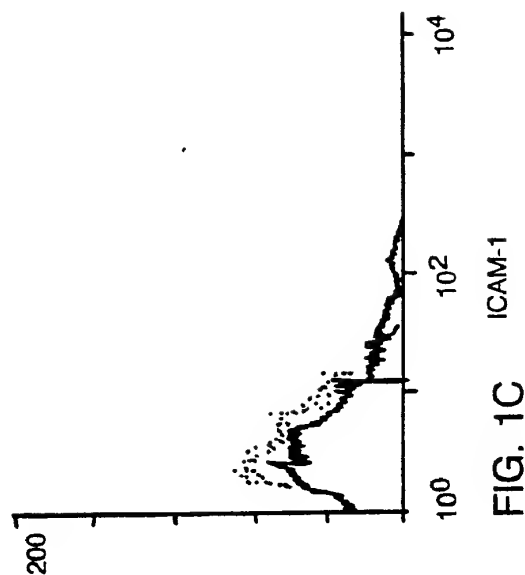
1           35. A method for inhibiting rejection by a  
2 recipient animal of a transplanted tissue containing  
3 cells bearing a surface antigen capable of causing a T-  
4 lymphocyte-mediated response in said animal via a  
5 receptor molecule on

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6 said T-lymphocyte of said animal, said method comprising  
7 transfecting said cells with DNA encoding a secretable  
8 protein or peptide capable of binding to said receptor  
9 molecule to competitively inhibit binding of said T-  
10 lymphocyte to said cells of said tissue via said  
11 receptor.

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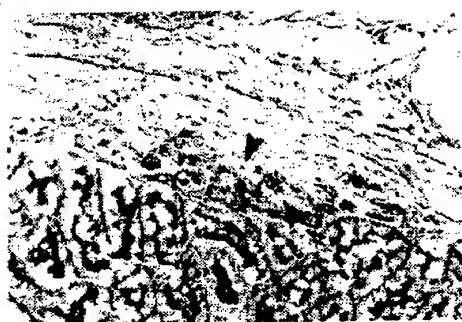


FIG. 2A



FIG. 2C

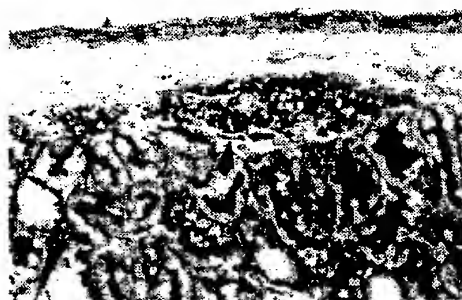


FIG. 2B

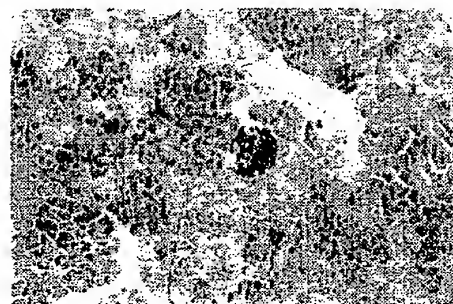


FIG. 2D



# INTERNATIONAL SEARCH REPORT

International Application No. PCT/US91/06105

<b>I. CLASSIFICATION OF SUBJECT MATTER</b> (if several classification symbols apply, indicate all) <sup>6</sup>		
According to International Patent Classification (IPC) or to both National Classification and IPC		
IPC(5): A61K 35/00 35/23, 37/00		
USCL.: 424/85.8, 572		
<b>II. FIELDS SEARCHED</b>		
Minimum Documentation Searched <sup>7</sup>		
Classification System	Classification Symbols	
U.S.	424/85.8, 572	
Documentation Searched other than Minimum Documentation to the Extent that such Documents are Included in the Fields Searched <sup>8</sup>		
Databases: Dialog UFiles 5,399,173,154,155,357), USPTO Automated Patent System (File USPAT, 1971-1991).		
<b>III. DOCUMENTS CONSIDERED TO BE RELEVANT <sup>9</sup></b>		
Category <sup>1</sup>	Citation of Document, <sup>11</sup> with indication, where appropriate, of the relevant passages <sup>12</sup>	Relevant to Claim No. <sup>13</sup>
Y	Science. Volume 204. issued 20 April 1979. Lacy, et al.. "Prolongation of Islate allograft Survival Following In vitro Culture (24°C) and a Single Injection of ALS". pages 312-313. see entire document.	1-20
Y	Proceedings National Academy of Sciences Volume 78, no. 8. issued August 1981. Farstman, et al.. "Prolognation of Murine Islet Allograft Survival Pretreatment of Islets with Antibody Directed to Ia Determinants, pages 5156-5159. see abstract.	1-20
<p><sup>10</sup> Special categories of cited documents:</p> <p>"A" document defining the general state of the art which is not considered to be of particular relevance</p> <p>"E" earlier document but published on or after the international filing date</p> <p>"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)</p> <p>"O" document referring to an oral disclosure, use, exhibition or other means</p> <p>"P" document published prior to the international filing date but later than the priority date claimed</p> <p>"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention</p> <p>"X" document of particular relevance: the claimed invention cannot be considered novel or cannot be considered to involve an inventive step</p> <p>"Y" document of particular relevance: the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.</p> <p>"Z" document member of the same patent family</p>		
<b>IV. CERTIFICATION</b>		
Date of the Actual Completion of the International Search	Date of Mailing of this International Search Report	
18 October 1991	18 DEC 1991	
International Searching Authority	Signature of Authorized Officer	
ISA/US	Lynette F. Smith	

III. DOCUMENTS CONSIDERED TO BE RELEVANT (CONTINUED FROM THE SECOND SHEET)		
Category *	Citation of Document, with indication, where appropriate, of the relevant passages	Relevant to Claim No
Y	Proceedings National Academy of Sciences Volume 86, issued November 1989. Koller et al., "Inactivating the B <sub>2</sub> -microglobulin Locus in Mouse Embryonic Cell by Homologous Recombination." pages 8932-8935. see abstract.	8.35
Y	EP.A. 0,314,863 (Anderson, et al.) 10 May 1989. see page 3.	19-20
Y	US.A. 4,009,257 (Thomas et al.) 22 February 1977, see entire document.	21-26
Y	US.A. 4,167,945 (Gottlieb) 18 September 1979. see entire document.	21-34
Y	US.A. 4,399,123 (Oliver, et al.) 16 August 1983, See entire document.	21-34
Y	Nature, volume 330, issued 10 December 1987. Doetschman, et al., "Targetted correction of a mutant HPRT Gene in Mouse Embronic stem cells". pages 576-578, see page 577.	6.35